179

The Consequence of Incorporation of (S)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine by Human Cytomegalovirus DNA Polymerase on DNA Elongation. X. F. Xiong, J. L. Smith, and M. S. Chen. Gilead Sciences, Foster City, CA 94404

(S)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl)cytosine (HPMPC, Cidofovir), an acyclic cytosine nucleotide analog with potent in vitro and in vivo activity against a broad spectrum of herpesviruses has been show to exert a dose-dependent anti-CMV effect as measured in the urine and semen of advanced AIDS patients. HPMPC diphosphate (HPMPCpp), the putative antiviral metabolite of HPMPC, is an inhibitor of HCMV DNA polymerase with a Ki value of 6.6 μM and competitive with respect to dCTP incorporation into activated calf thymus DNA. HCMV DNA polymerase uses synthetic DNA efficiently with Km value of 0.09 μM. The Km values for HPMPCpp and dCTP in using synthetic DNA are 13.2 and 0.51 μM, respectively. The ratio of Vmax/Km values of HPMPCpp to dCTP is 0.056 with synthetic DNA as the primer-template. HCMV DNA polymerase can also use HPMPC terminated primers annealed to synthetic DNA templates with a Km value of 0.165 μM. The incorporation of one HPMPC molecule causes the DNA elongation to slow down for the addition of the next two natural nucleotides. The addition of the second natural nucleotide away from the incorporated HPMPC is slower than the addition of the first nucleotide. The overall slow down in DNA synthesis by one incorporated HPMPC is 31%. However, the fidelity of HCMV DNA polymerase is maintained for the addition of nucleotides after a singly incorporated HPMPC. The rate of further elongation by HCMV DNA polymerase is undetectable after the incorporation of two consecutive HPMPC molecules. The incorporation of two HPMPC molecules separated by one nucleotide (dAMP, dGMP,or dTMP) also abolishes DNA chain elongation by HCMV DNA polymerase. Incorporation of two HPMPC molecules separated by two nucleotides allows DNA chain elongation to continue. The DNA synthesis by HCMV DNA polymerase slows down 64% when using DNA template that contains one internally incorporated HPMPC molecule. These data indicate that HPMPC exerts diverse inhibitory mechanisms on the replication of HCMV DNA synthesis.

180

Specific Anti-Cytomegaloviral Activity of Methotrexate Associated with Preferential Accumulation of Drug by Infected Cells. M. Wachsman, F.M. Hamzeh, H. Saito, and P. S. Lietman. [1] Division of Clinical Pharmacology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD

We have previously shown that inhibitors of pyrimidine biosynthesis including azaserine and pyrazofurin, were active against human cytomegalovirus (HCMV) (Abstract # 129 ISAR 1993). We extend these observations with the thymidine biosynthesis inhibitor methotrexate, and demonstrate specific activity against cytomegalovirus replication correlating with increased intracellular accumulation and polyglutamation of methotrexate in cytomegalovirus infected cells. The IC50 of methotrexate for inhibition of cytomegaloviral DNA replication at 3 days post infection (p.i.) in MRC-5 cells was 4 x 10^{-8} M (0.04 μMolar) and no cell toxicity was observed in uninfected confluent cells at the highest concentration tested (1 μMolar). Under similar conditions, (3 days of treatment with the IC90 of methotrexate (0.4 μMolar)) deoxythymidine triphosphate concentrations were diminished in cytomegalovirus infected cells (87% decrease relative to untreated infected cells $p < 0.001$) and not in uninfected cells. A potential explanation of the specific anti-viral effect of methotrexate was preferential accumulation of methotrexate by HCMV infected cells. Increased uptake of [^{3}H]-methotrexate by cytomegalovirus infection was first observed at 48 hrs. post infection with 3 fold higher accumulation in infected cells. This preferential accumulation increased to approximately 4 fold at the end of the observation period (96 hrs). The uptake of [^{3}H]-methotrexate was saturable and was blocked by addition of unlabelled methotrexate. The intracellular methotrexate in infected cells was almost entirely the polyglutamated form as demonstrated by thin layer chromatography while both unmetabolized and polyglutamated forms were demonstrable in the uninfected cells.

HCMV Infection	Methotrexate Treatment	dTTP ± SD (pMol/10^6 Cells)
-	-	0.38 ± 0.11
-	+	0.52 ± 0.04
+	-	3.14 ± 0.44
+	+	0.39 ± 0.05